PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/005208	14.05.2004	15.05.2003

International Patent Classification (IPC) or both national classification and IPC
G01N33/566, G01N33/569, G01N33/50, C07K16/00

Applicant
CYTOS BIOTECHNOLOGY AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/005208

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 58

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 58

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☒ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1,2,5,7-46,49,51-57,59-62

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5,7-11,37-42,44-46,49,51-57,59,60
	No:	Claims	1,2,12-36,43,61,62
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1,2,5,7-46,49,51-57,59-62
Industrial applicability (IA)	Yes:	Claims	1,2,5,7-46,49,51-57,59-62
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item III:

The subject-matter of claim 58 belongs to the reach through claim format and was thus not searched. No opinion will be given on subject-matter that has not been searched.

Re Item IV:

The requirements of unity of invention (Rule 13 PCT) are not fulfilled in that there is no technical relationship among the inventions as they do not involve one or more of the same or corresponding special technical features.

Claim 1 refers to a method for selecting at least one antigen-specific B cell from a mixture of cells comprising: a mixture of cells comprising B cells, a first composition comprising a core particle, at least one antigen, wherein the antigen and the core particle form through association an ordered and repetitive antigen array, contacting the first composition with said mixture of cells, labelling the composition and the B cells independently and selecting a B cell which is positive for said first and second labelling compound. Claim 2, refers to the different core particles used: a virus, a virus-like particle, a bacteriophage, a bacterial pilus, a viral capsid particle and a virus-like particle of a RNA-phage. According to Rule 13 PCT a group of inventions is considered to be unitary only if so linked as to form a single general inventive concept. The sole concept linking independent claim 1 and the different core particles of claim 2 is the use of said core particles for the isolation of antigen-specific B-cells by forming an ordered and repetitive antigen array. However, the use of virus-like particles for the isolation of antigen-specific B-cells is already known in the art.

Thus, in the light of the prior art, the problem to be solved by the present application can be formulated as follows: How to isolate further antigen-specific B-cells by core particles forming an ordered and repetitive antigen array. The solution to this problem is a composition comprising a core particle and an antigen according to present claim 1.

However, the use of a Rotavirus derived virus-like particle for the isolation of Rotavirus-specific B-cells is known from the prior art (see J. Immunol. Methods (D1), vol. 275, 2003, pg.: 223-237, abstract, page 224, col. 2, second para. to page 225, col. 1, para. 1, figure 1; page 235, col. 1, third para. to page 236, col. 1, second para.). D1 is also detrimental to the novelty of the concept of an ordered antigenic composition array as

referred to in claim 1 for the following reasons:
Claim 1 refers broadly to a composition comprising a "core particle" and an antigen. D1 discloses on page 224, second col., second paragraph that the RV virus-like particle consists of recombinantly produced proteins VP2, VP6 and VP7 wherein VP6 and VP7 are strongly antigenic in contrast to VP2. VP2 must therefore be covered by the two other antigenic proteins meaning that it acts as a "core or nucleation particle" for the two other antigens. It is pointed out that neither the term "core particle" nor the term "antigen" is defined in claim 1 and have therefore to be broadly interpreted. Thus, the RV VLPs of D1 are at the same time "core particles" according to claim 2 as well as a composition according to claim 1 forming a highly repetitive antigenic structure comprising a core particle and an antigen. Additionally, the application itself uses a Hepatitis B core antigen being a single protein as a "core particle" (see example 1 of the present application). All the other method steps of claim 1 are disclosed in D1 starting from page 225 fig. 1 and col. 2, second para. to page 226, col. 1, last para.). Thus, D1 is clearly detrimental to the novelty of the concept of present claim 1. Consequently, all the other core particles mentioned in claim 2 constitute separate alternative solutions to the problem mentioned above. Thus, there is no longer a technical relationship between the six core particles mentioned above.

Thus, the following groups of inventions have to be discerned:

Group I: Claims 1, 2, 10 to 46, 54 to 62(all partially) referring to virus core particle.

Group II: Claims 1,2(all partially), 3, 4 (both complete), 5(partially), 6(complete), 10 to 46(all partially), 47, 48 (both complete), 49(partially), 50(complete), 54 to 62(all partially) referring to virus-like core particle.

Group III: Claims 1, 2, 10 to 46, 54 to 62(all partially) referring to bacteriophage core particle.

Group IV: Claims 1, 2, 10 to 46, 54 to 62(all partially) referring to a core particle consisting of a bacterial pilus.

Group V: Claims 1, 2, 10 to 46, 54 to 62(all partially) referring to viral capsid core particle.

Group VI: Claims 1,2(all partially), 5(partially), 7 to 9(all complete), 10 to 46(all partially), 49(partially), 51 to 53(all complete), 54 to 62(all partially) referring to a core particle consisting of a virus-like particle of a RNA phage.

Since the applicant has paid for group I and group VI invention, the following opinion will be restricted to the subject-matter of said inventions.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:
D1: J. Immunol. Methods, vol. 275, 2003, pg.: 223-237
D2: WO-A-03024481
D3: Vaccine, vol. 20, 2002, pg.: 3104-3112
D4: Immunology Today, vol. 17, 1996, pg.: 553-557
D5: US-A-5326696

1. Novelty

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1, 2, 12 to 36, 43, 58, 61 and 62 is not new in the sense of Article 33(2) PCT.

The document D1 discloses the method of claim 1 by using a fluorescence labelled Rotavirus core virus-like particle for the isolation of Rotavirus specific B-cells and antibodies. Since core particles consisting of different structure proteins are used and different structure protein specific B-cells and antibodies are isolated, D1 is considered to be detrimental to the novelty of the subject-matter of claims 1, 2, 12 to 36, 43, 58, 61 and 62 (see abstract, page 224, col. 2, second para., fig. 1, page 226, col. 1, third para. to page 227, col. 2, first para., page 231, col. 1, second para. to col. 2, first para.; page 235, col. 1, third para. to page 236, col. 1, second para).

2. Inventive step

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1, 2, 5, 7 to 46, 49 and 51 to 62 does not involve an inventive step in the sense of Article 33(3) PCT.

The document D1 is regarded as being the closest prior art. It discloses the method of present claim 1. The subject-matter of claims 10 and 54 therefore differs from this known document in that the association between the antigen and the core particle is through at

least one covalent bond. D1 discloses only a spontaneous association of the different structural protein by non-covalent bonds. However, this difference is considered to be arbitrary not resulting in any unexpected or surprising technical effect. It does not mention VLPs from RNA phages. Moreover, D2 teaches already recombinant "viral core particles" containing covalently bound foreign antigens for the induction of strong immune response in B cells by binding to and cross linking the specific surface B-cell receptor (see page 6, first para., page 26, fourth para.). This refers to virus and RNA phage core particles, such as Hepatitis B virus and Qβ phage (see page 28, lines 6 to 15, page 36, third para., to page 45, last line.). Moreover, D3 and D4 teach that in particular the highly repetitive and organised array on viral capsid surfaces lead to a strong cross-linking of B cell receptors which results in a highly specific ans selective B cell response (see D3, page 3104, col. 2, second para. to page 3105, col. 1, first para.; D4, page 553, col. 1, first para. to col. 2, first para.). Thus, the person skilled in the art would have combined the teaching of D1 with either D2, D3 or D4 to arrive at the claimed subject-matter falling within the scope of claim 5, 7 to 10, 49, 51 to 53 and 54 without employing any inventive skill. Consequently, the subject-matter of present claim 2 does not appear to be inventive and does not fulfil the requirement of Article 33(3) PCT.

The same reasoning applies, mutatis mutandis, to the subject-matter of claims 37 to 40 referring to a third label which appears not to result in any unexpected or surprising technical effect (moreover, it is known from US-A-5326696, abstract) or the use of splenocytes as a source of B-cells instead of peripheral blood cells since it is well known that B-cells occurs in large amounts in the spleen (claims 42, 44 to 46). The same applies to the fusion methods of claims 59 and 60 which are standard methods in the art.